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During the last funding period, we produced high-titer recombinant AAV vectors encoding mouse endostatin and angiostatin, and human osteoprotegerin, established TRAMP mouse breeding colony, and initiated in vivo studies to determine the effects of anti-angiogneic therapy at two different stages of prostate cancer progression. Additionally, we constructed rAAV encoding human OPG, produced high-titer virus and validated the biological efficacy of the vector encoded protein in inhibiting osteoclastogenesis in vitro. Continuation of the ongoing studies in to next year will provide valuable information on therapeutic effects of anti-angiogenic gene therapy using adeno-associated virus in prostate cancer growth and metastasis.

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INTRODUCTION

One of the major implications of prostate cancer progression is bone metastasis. Primary therapies for neoplastic prostate disease have been prostatectomy followed by chemotherapy and radiation therapy. Although these forms of palliative therapies have been successful in early detected prostate cancers, a problem in majority of the treated cases is the growth of radiation/chemotherapy resistant tumor cells, which become refractory to treatment and exhibit an aggressive growth and metastatic profile. Thus, novel therapies that will control the process of recurrence and metastasis will have a profound clinical implication in the management of prostate cancer patients who undergo primary therapies.

An interesting new target for prostate cancer therapy is tumor angiogenesis, which is vital for tumor growth and metastasis. Since anti-angiogenic therapy targets normal endothelial cells that form neovasculature, long term sustained presence of anti-angiogenic factors is critical for therapeutic significance. Although few drugs and purified proteins have shown preclinical efficacy of this form of therapy, a long-term application of these therapies have been associated with systemic toxicity, limited half life and increasing cost. Thus, stable long-term therapies without these effects would be highly beneficial. Gene therapy approach using recombinant adeno-associated virus vectors (rAAV) encoding anti-angiogenic factors is a very promising form of therapy for prostate cancer recurrence and metastasis. Major advantage of rAAV vectors are 1) long-term transgene expression 2) stable integration, 3) low-immunogenicity or toxicity and 4) non-pathogenicity.

Our recent preclinical evaluation using rAAV encoding angiostatin, endostatin and soluble vascular endothelial growth factor receptor (sFlt-1) indicated long-term protection of mice against the growth of a human angiogenesis-dependent ovarian cancer cells as xenograft. Sustained expression of the anti-angiogenic factors was detected over four months without any systemic toxicity. Based on these data, we proposed in our funded application to evaluate the potential of rAAV-mediated anti-angiogenic gene therapy in a transgenic adenocarcinoma mouse prostate (TRAMP) model, which exhibits most of the pathological features seen in human prostate cancer including a progressive angiogenic phenotype with advancing stages of the disease, bone metastasis and refractiveness of androgen depletion over time. New experiments will include the analysis of bone metastasis of prostate cancer cells following rAAV-mediated anti-angiogenic gene therapy. Further, we will also determine the effects of long-term expression of murine osteoprotegrin as primary and an adjuvant to anti-angiogenic gene therapy for the inhibition of bone metastasis of malignant prostate disease in mouse model.

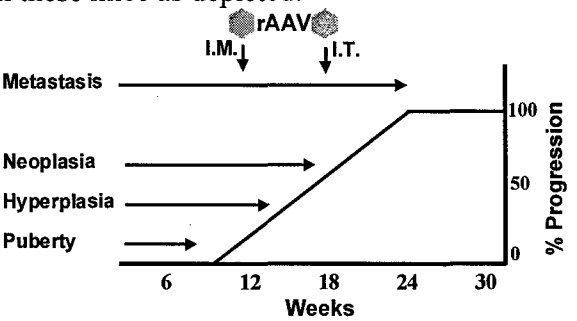
The proposed specific aims of the project are:

1. To determine long-term therapeutic potential of rAAV-mediated anti-angiogenic gene therapy in bone metastasis of neoplastic prostate disease in the transgenic adenocarcinoma mouse prostate (TRAMP) model *in vivo*.
2. To determine the adjuvant effects of long-term anti-angiogenic gene therapy and osteoprotegrin therapy for androgen-independent recurrence of prostate cancer in the TRAMP model.

BODY

Determination of rAAV-mediated anti-angiogenic gene therapy for early and late stage prostate cancer in mouse model.

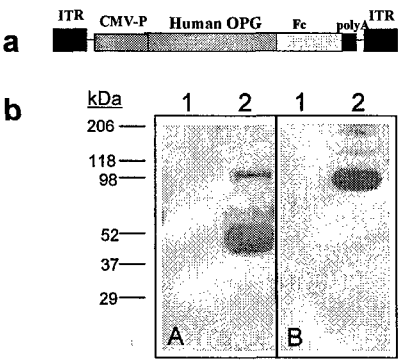
During the last year, we have produced high-titer recombinant AAV containing mouse angiostatin and endostatin and initiated *in vivo* studies in male transgenic adenocarcinoma of prostate mouse (TRAMP) model. We have developed a breeding program for obtaining sufficient male TRAMP mice for the studies. The in vivo studies have been initiated at two different phases of prostate cancer development in these mice as depicted.



Treatment regimen with rAAV encoding angiostatin and endostatin in TRAMP mice for early and late-stage disease.

These long-term studies are ongoing and we will have conclusive data in approximately 3 months. However, based on the ongoing trend, it appears that rAAV-mediated anti-angiogenic gene therapy may provide significant therapeutic benefit when administered during early stage disease. Although marginal therapeutic gains are noted in the group treated with rAAV encoding angiostatin and endostatin during 18-weeks of age, the reduction in tumor growth was not dramatic.

Construction of rAAV encoding human OPG and analysis of expression as a soluble factor. To determine if rAAV-mediated gene therapy can be used to inhibit prostate cancer bone metastasis, a rAAV containing the N-terminal 185 amino acid portion of the human OPG cDNA fused to the human immunoglobulin (Fc) was constructed. The construct was tested for the expression and extracellular secretion of OPG in RAW (a murine macrophage cell line) cell cultures. Results, shown below, indicate the expression of OPG from rAAV transduced cells.

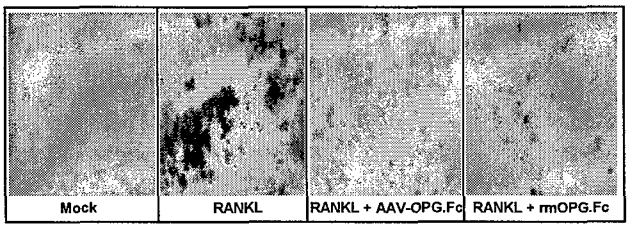


Recombinant AAV encoding the human OPG.Fc (a) and expression of the OPG.Fc from RAW cell supernatant (b). RAW cells were mock-transduced (1) or transduced with rAAV-OPG.Fc (2) construct and the supernatants were analyzed by Western blot using a monoclonal antibody for the human OPG in either denartured gel (A) or non-denatured gel (B).

Transduction of rAAV-OPG.Fc inhibits osteoclast differentiation *in vitro*. The biological activity of rAAV produced OPG was determined in osteoclast forming assay using RAW cells. Briefly, 10^5 RAW cells were plated in 24-well tissue culture plates and grown in medium containing 10% FBS, 20 ng/ml M-CSF, and 50 ng/ml RANKL in the presence or absence of conditioned medium from 293 cells transduced with rAAV-OPG.Fc. The growth medium plus

additives were changed every alternate day. After five days of culture, the cells were fixed and stained for tartrate-resistant alkaline phosphatase (TRAP), a marker for multinucleated osteoclasts. Results, shown below, demonstrate that rAAV produced OPG is biologically active and effectively inhibits osteoclastogenesis.

TRAP assay of RAW cells following rAAV-OPG.Fc transduction.



In the next year, we will test the efficacy of rAAV-OPG vector in inhibiting the initial osteolytic degradation, which leads to osteoblastic lesions in prostate cancer. These will be done in a SCID mouse model instead of TRAMP model since the bone metastasis occurs only in ~25% of the TRAMP mouse.

KEY RESEARCH ACCOMPLISHMENTS

- Produced high-titer recombinant AAV vectors encoding mouse endostatin and agniostatin, and human osteoprotegerin.
- Established TRAMP mouse breeding colony.
- In vivo studies to determine the effects of anti-angiogneic therapy at two different stages of prostate cancer have been initiated.
- Constructed rAAV encoding human OPG, produced high-titer virus and validated the biological efficacy of the vector encoded protein in inhibiting osteoclastogenesis in vitro.

REPORTABLE OUTCOMES

(Papers published or communicated)

Isayeva, T., and Ponnazhagan, S. Anti-angiogenic gene therapy for cancer. Int. J. Oncol. 2004, 25: 335-343.

Mahendra, G., Mahasreshti, P., Curiel, D.T., Stockardt, R., Grizzle, W.E., Alapati, V., Singh, R., Siegal, G.P., and Ponnazhagan, S. Anti-angiogenic gene therapy through adeno-associated virus 2-mediated stable expression of soluble Flt-1 receptor Cancer Gene Ther. 2005, 12: 26-34.

Isayeva, T., Ren, C., and Ponnazhagan, S. Recombinant adeno-associated virus 2 -mediated anti-angiogenic prevention in a mouse model of intraperitoneal ovarian cancer. Clin. Cancer Res. 2005 11:1342-1347.

(Results presented in conferences)

Isayeva, T., Ren, C. and Ponnazhagan, S. Intraperitoneal transduction of adeno-associated virus 2 expressing angiostatin and endostatin synergistically augments paclitaxel therapy and tumor-free survival in a mouse model of epithelial ovarian cancer. *96th Annual Meeting of the American Society for Cancer Research*, Anaheim, CA. April 2005

CONCLUSIONS

During the last funding period, we produced high-titer recombinant AAV vectors encoding mouse endostatin and angiostatin, and human osteoprotegerin, established TRAMP mouse breeding colony, and initiated in vivo studies to determine the effects of anti-angiogneic therapy at two different stages of prostate cancer progression. Additionally, we constructed rAAV encoding human OPG, produced high-titer virus and validated the biological efficacy of the vector encoded protein in inhibiting osteoclastogenesis in vitro. Continuation of the ongoing studies in to next year will provide valuable information on therapeutic effects of anti-angiogenic gene therapy using adeno-associated virus in prostate cancer growth and metastasis.

PERSONNEL RECEIVING PAY FROM THIS GRANT

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REFERENCES

N/A

APPENDICES

N/A